

mesenchymal markers, vimentin and N-cadherin in STM cells. Enhanced invasion and migration capabilities were observed in STM cells consistent with a mesenchymal phenotype. Src has been shown to play a role in E-cadherin regulation and EMT. Thus, treatment of cells with dasatinib, a Src inhibitor, suppressed cell growth in STM cells, but not in ALK+/TKI sensitive cell lines. The addition of a low dose of dasatinib sensitized STM cells to the anti-proliferative effects of crizotinib. STM cells were subjected to genetic analysis to identify new genetic anomalies that could be driving resistance. Top hits are being evaluated. To our knowledge, this is the first report that demonstrates EMT occurring in an ALK+ crizotinib resistant clinical sample. Collectively these data support EMT as a mechanism of resistance to crizotinib and identifies dasatinib as a potential therapeutic for treatment of crizotinib resistance associated with EMT.

CD3+ cells displaying the activation antigens HLA-DR+/CD38+. PD-L1 positive tumors also have a significantly higher proportion of FOXP3+/CD4+ regulatory T cells. We found that CD4+ and CD8+ T cells in PD-L1 positive samples are significantly more likely to express the T-cell exhaustion markers PD-1 and TIM-3 compared to PD-L1 negative samples. Flow cytometric analysis also identified two immunologically distinct PD-L1 positive mesothelioma samples: PD-1 positive, TIM-3 negative “single positive” tumors, and PD-1 positive, TIM-3 positive “double positive” tumors. Successful incorporation of comprehensive immune profiling by flow cytometry into prospective clinical trials should hopefully refine our ability to predict which patients will respond to immune checkpoint blockade and lead to rationally designed combination immunotherapy trials.

### Immune profiling of malignant pleural mesothelioma by flow cytometry identifies distinct T-cell activation and exhaustion phenotypes in PD-L1 positive versus PD-L1 negative tumors



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Although PD-L1 immunohistochemical staining appears to be a partially predictive biomarker of response to PD-1 inhibitors in some cancers, many PD-L1 positive tumors do not respond to these agents. Possible explanations for this observation are that some PD-L1 positive cancers may have a paucity of infiltrating lymphocytes and/or T cells within tumors may express multiple exhaustion markers. We have developed a method for comprehensive immune cell phenotyping using flow cytometry on solid tumors that have been dissociated into single cell suspensions. Applying this technique to 33 resected malignant pleural mesothelioma samples, here we show that compared to PD-L1 negative tumors, PD-L1 positive tumors have significantly more infiltrating CD45+ immune cells, a significantly higher proportion of infiltrating CD3+ T cells, a significant increase in proliferating CD4+ and CD8+ T cells, and a significantly higher percentage of

### Novel epidermal growth factor receptor inhibitor accumulates in the brain and inhibits the growth of brain metastatic non-small cell lung cancer



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Deaths from solid tumors are often not due to the primary lesion but to metastatic disease at distal sites such as the lung, liver, and brain. Patients with non-small cell lung cancer (NSCLC) experience brain metastases, a poor prognostic marker, at an incidence rate of 30-50%. A significant proportion of the metastatic tumors express activating mutations of the EGFR, including exon 19 deletions, which confer increased sensitivity to EGFR inhibitors such as erlotinib and gefitinib. Despite successful use of small molecule kinase inhibitors for the treatment of EGFR+ primary lung tumors, current therapeutics poorly inhibit the growth of NSCLC brain metastases due to difficulty crossing the blood-brain barrier. For this reason, NSCLC patients with brain metastases are often excluded from clinical trials with novel therapies and thus have access to very few emerging treatment options.

We have synthesized a novel class of compounds that inhibit the epidermal growth factor receptor (EGFR) in the nanomolar range in vitro, and demonstrate a high degree of selectivity for EGFR family members. The parent compound, LL001, from Capella Therapeutics, Inc., inhibited EGFR-mediated autophosphorylation and